

Research article

Cooperation during prey digestion between workers and larvae in the ant, *Pheidole spadonia*

D.L. Cassill¹, J. Butler², S.B. Vinson² and D.E. Wheeler³

¹ Department of ESP-Biology, USF St. Petersburg, St. Petersburg, Florida 33701, U.S.A., e-mail: cassill@stpt.usf.edu

² Department of Entomology, Texas A & M University, College Station, Texas, U.S.A., e-mail: bvinson@neo.tamu.edu

³ Department of Entomology, University of Arizona, Tucson, Arizona 85721, U.S.A., e-mail: dewnants@ag.arizona.edu

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Abstract. Digestion and distribution of nutrients are central to the growth and reproduction of social insect colonies, just as they are to individual organisms. In the case of eusocial insect species, different components of food handling and processing can be distributed among castes. This paper reports on an ant species, *Pheidole spadonia*, in which the adult workers butcher prey and 4th instar larvae dissolve prey for distribution among other colony members including workers, larvae and queens. To characterize the process, six groups, each composed of twenty-five workers and thirty larvae, were provisioned with a fruit fly carcass, and then video-taped continuously for 24 hours. On average, five adult workers and twenty-two 4th instar larvae invested 12.8 labor hours into butchering and predigesting one fly carcass. Workers contributed a mean total of 3.3 labor hours to butcher the carcass into small fragments. Fourth instar larvae contributed a mean total of 9.5 labor hours to pre-orally dissolve the solid fragments. Surprisingly, larvae did not ingest during the dissolving process. Instead, workers ingested the dissolved prey tissue into their crops and then regurgitated it to colony members, larvae and workers, that solicited for feedings. The cooperative interactions reported here between workers and larvae extend the mechanistic and evolutionary explanations for eusociality.

Keywords: Diet, nutrition, feeding behavior, parental care, mutualism, division of labor.

Introduction

Although the flow of liquid food among ant larvae and workers has been well characterized, primarily in fire ants (Wilson and Eisner, 1957; Sorensen and Vinson, 1981; Sorensen et al., 1985; Cassill and Tschinkel, 1995, 1999a,b), the flow of prey tissue has not. In the few cases when the handling

of prey tissue has been examined, it accounted for a very small percentage of feedings – fewer than 1% of feedings in *Solenopsis invicta* (meal worm larvae, Cassill and Tschinkel, 1999c) and 4% of larval feeding events in *Myrmica rubra* (trophic eggs and insect larvae, Kipyatkov and Lopatina, 1989). The apparent rarity of feeding prey to larvae is puzzling since a large proportion of the diet of most ants is insect and other arthropod tissue (Ayre, 1963; Sudd, 1983; Hölldobler and Wilson, 1990; Tennant and Porter, 1991). Moreover, protein in large quantities is a requirement for insect growth (Brian, 1983; Raubenheimer and Simpson, 1997; Simpson and Bernays, 1983; Slansky and Rodriguez, 1987; Slansky and Wheeler, 1992).

As adults, aculeate Hymenoptera are not bulk feeders. Adults cannot pass large particles of prey tissue to the mid-gut because the narrow diameter of their petiole, through which the esophagus passes, restricts the size of particles that can transit through it (Glancey et al., 1981; Hölldobler and Wilson, 1990; Hunt, 1994). The fact that ants cannot move large pieces of prey tissue to their mid-guts, where it could be digested, coupled with the finding that ant mid-guts produce few if any proteases (Delage, 1968 as cited in Hölldobler and Wilson, 1990; Law et al., 1977; Petralia et al., 1980), has led to the hypothesis that, at least in higher ants, ant larvae function as the digestive caste for adult colony members (Wheeler, 1918; Went et al., 1972; Abbott 1978; Sorensen et al., 1983; Cassill and Tschinkel, 1999c).

To determine how insect prey carcasses are handled, we used colonies of the ant *Pheidole spadonia*. *Pheidole* is a large genus containing hundreds of species (Hölldobler and Wilson, 1990), most of which are little studied. A defining characteristic of *Pheidole* is dimorphism between the large-headed soldier caste and the minor worker caste. *Pheidole* thrive in the laboratory on a diet of insect flesh and honey water. This study of the processing of fruit fly carcasses, reported here, revealed the cooperative mechanisms by which

Pheidole colonies gains access to the proteinaceous nutrients in prey tissues.

Method and materials

Eight stock colonies of *P. spadonia* were reared from newly-mated queens captured in July of 1995 in Tucson, Arizona, U.S.A. Colonies were five years old at the time of this study. Stock colonies were maintained in incubators at 30°C and fed a diet of frozen, sliced *Manduca sexta* larvae and 30% honey water containing vitamins and minerals (see Bhatkar and Whitcomb, 1970). Six experimental groups were formed from three stock colonies. Each experimental group contained 25 minor workers and 30 4th instar larvae (Fig. 1). Eggs and younger instars were excluded from the study as it is known that younger instars do not contribute to the dissolving of prey tissue (Hölldobler and Wilson, 1990). Soldier workers were not included in this experiment because a preliminary study had shown that soldiers contribute little to larval care in experimental groups of this composition. Each group was maintained in an artificial nest with a glass cover.

Artificial nests were constructed of blocks of hydrostone dental plaster (4 × 4 × 2 cm) with a brood chamber (1.5 × 1 × 0.2 cm) molded into the dorsal surface. A glass cover slip sealed the brood chamber except for an entrance tunnel that allowed workers to move in and out of the nest chamber. The artificial nest was placed in a plastic petri dish (15 cm), which functioned as a foraging arena for workers. The inside walls of the petri dish were painted with Fluon[®] to prevent worker escape. The six experimental groups were deprived of food for 24 h prior to the initiation of this experiment.

Sugar water, 30% concentration, and one thawed fruit fly (*Drosophila melanogaster*) were placed in the arena of the artificial nest in the morning. Using video technology (Eyecom PSI 9000 with a Sony 18–108 mm lens set at 25× magnification; JVC recorder HR-A41U and a Sony 17" monitor), the entire brood chamber including workers and larvae was video-taped for 24 consecutive hours. We quantified and mapped the frequency and duration of fly carcass processing by workers and 4th instar larvae (Fig. 1) of each group until each fly was consumed.

Data were analyzed to obtain descriptive statistics using JMP IN statistical software, version 4.

Results

Qualitative description

Foraging ants retrieved flies from the foraging arena and carried them into the artificial nest chamber. Within seconds, the fly's wings were severed and discarded. The head and legs of the fruit fly were then severed, usually within minutes. The fly's head and legs were carried directly to a larva and placed on its food basket, the ventral or 'venter' region of the body that is morphologically specialized for holding food (Wheeler, 1918; Petralia and Vinson, 1979). In contrast, the fly's thorax and abdomen was butchered into smaller pieces. Workers, often working in groups of two or three, began the process by pulling against each other as they chewed and slashed the carcass with their mandibles. Workers licked and ingested body fluids, then masticated the carcass into smaller, drier fragments.

Several times during the butchering process, workers placed the fly carcass briefly onto the food basket of larvae – from 30 s to 120 s. During this time, a larva would coat the carcass with saliva as the worker intervened to rotate the

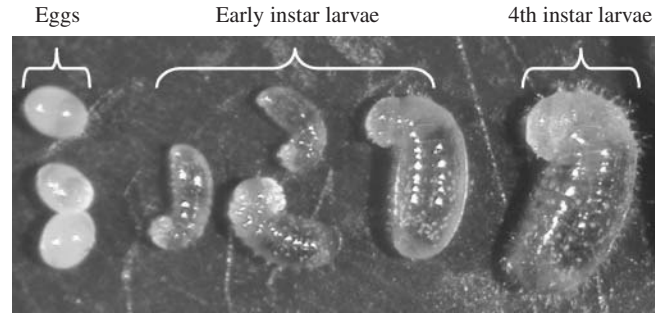


Fig. 1. Eggs and the four larval instars of the harvester ant, *Pheidole spadonia*. Only 4th instar larvae participated in the dissolving/digesting of prey tissue. Photo by Norm Buck.

carcass and “test” it by quick licks with the glossae. Based on some cue, perhaps the softness of the prey cuticle, workers removed the fly carcass and continued to masticate it into still smaller fragments. This process of butchering and dehydration by workers and softening and dissolving by larvae continued until the carcass was divided into three or four fragments.

Once the prey was butchered, workers positioned prey fragments using their mandibles and forefeet to tamp it into place onto the food baskets of larvae, just under the mandibles of the larvae. After placement, workers moved away and the larvae began an extended process of piercing the fragment with their slender, sclerotized mandibles. Piercing the fragment increased its surface area by creating many small pockets into which digestive enzymes moved. Workers regularly patrolled larvae, contacting them on average, every 4 s to 10 s. Workers assessed the prey fragment by giving it a quick lick with the glossae or by picking up the fragment with their mandibles, licking it briefly for one or two seconds and then depositing it back on the larva's food basket. As the fragment began to dissolve, workers removed it from the larva's food basket for an extended period of 10 s to 45 s, licked and ingested the dissolved portion until the tissue was reduced to a smaller, hard fragment. Then workers replaced the newly reduced fragment onto a larva's food basket – sometimes the same larvae, more often a different larva (Fig. 2). After a partially dissolved fragment was removed from a larva, that larva received extensive grooming around the mouthparts and food basket from other workers. This cycle of fragment dissolving by larvae and mastication (chewing), licking and shrinking by workers was repeated until each prey fragment was completely dissolved by larvae and ingested by workers.

Quantitative description

On average, 12.8 labor hrs (range = 6.9–15.1 hrs) were required to butcher, digest and ingest one fruit fly carcass. Because each fruit fly carcass was butchered into two to four fragments and rendered concurrently by multiple individu-

als, the real time to butcher and digest a carcass was 3–5 hrs. On average, each fruit fly carcass was handled a mean total of 70 times (range = 55–118) by 22 larvae. The distribution of larval labor was skewed, with ~ 29 % of the 4th instar larvae contributing 60 % of the labor (Fig. 3). The median total digestion time per larva was 20.5 min.

The distribution of worker labor toward the butchering of fly carcasses was skewed as well. A mean of five of the twenty-five workers in the nest contributed to the butchering of a fly carcass. On average, each of the five workers con-

tributed forty (40) labor minutes to the butchering process, rendering each fly carcass into two to four fragments. Each worker handled a mean total of 5.7 fly carcass fragments for a mean total of 7 minutes per fragment.

In summary, a mean 20% of the worker force (5/25 workers per group) labored a total of 3.3 hours, contributing 25.8% of the total labor needed to butcher a single fly carcass into two to four shriveled fragments. A mean 73.3% of the larval group (22/30 larvae) labored a total of 9.5 hours, contributing 74.2% of the mean total labor hours to dissolve a fruit fly. Although each worker labored 50% longer per fly carcass than each larva (~ 40 minutes versus ~ 20 minutes per fragment), the number of workers was far smaller than the number of larvae laboring to digest a fly carcass.

To determine the rate of ingestion and swallowing by larvae as they dissolved a fly carcass, twenty larvae were observed for five consecutive hours with a dissecting microscope at 25× to 50× magnification. To enhance the visibility of boluses swallowed by the translucent larvae, fly carcasses were dyed using over-the-counter food coloring (McCormick’s). Surprisingly, ingestion by larvae while dissolving prey carcasses was rare. Only a few larvae ingested. All together, larvae ingested only 1.7% of the time that they spent dissolving the fly carcass—a negligible amount. During those rare ingestion events, the mean duration of ingestion was 13.4 s (range = 2–28 s); the mean rate of ingestion was 2.6 boluses / s.

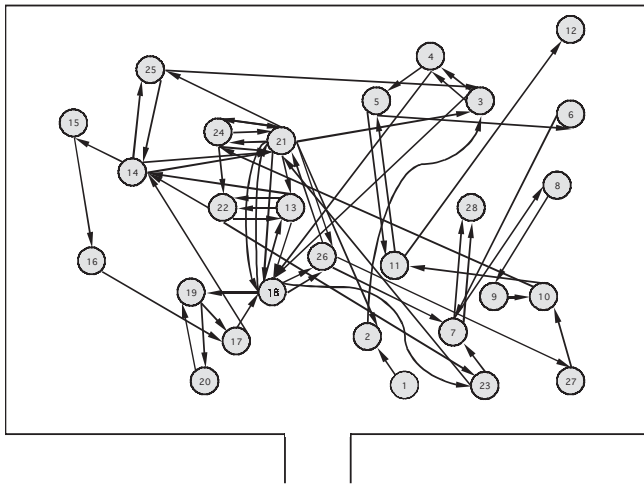


Fig. 2. One of six maps showing the location of larvae (circles) in the artificial nest and the sequential movement of butchered pieces of a fruit fly carcass among them (number in the circles). The arrowed lines indicate the order of flow of the prey fragments among larvae.

Discussion

In our study of the distribution of protein in *Pheidole spadonia*, we found four distinct phases of digestion and ingestion. First, workers butchered prey tissue into small fragments and licked the tissue, presumably ingesting body fluids and hemolymph, until the prey was dehydrated. This is the mastication phase. Second, workers anchored each fragment onto a larva’s food basket. Third, larvae dissolved the fragments externally using saliva and mastication to saturate the prey fragment with enzymes. Fourth, workers repeatedly tested the fragment with their glossae until it was further or entirely dissolved. When the tissue was sufficiently dissolved, workers ingested the fluid portion from the larval food basket. Workers distributed the dissolved prey tissue to larvae and other workers. For prey tissue, larvae are the colony’s social stomach. When dissolved, the fragment had a shiny liquid appearance that could have been mistaken for saliva. Perhaps previous studies that observed larvae offering salivary products to workers were instead observations on the product of an extended pre-oral digestive process of prey tissue by larvae.

Similar steps in processing of arthropod prey have been described for wasps in the context of social evolution. Hunt (1994) argued that mastication of arthropod prey, larval-adult trophallaxis and inter-adult trophallaxis are feeding behaviors that have contributed to the repeated evolution of eusociality in wasps, as well as other Hymenoptera. In wasps, workers and larvae share and at times compete for prey tissue. In *P. spadonia*, however, the mutualism among

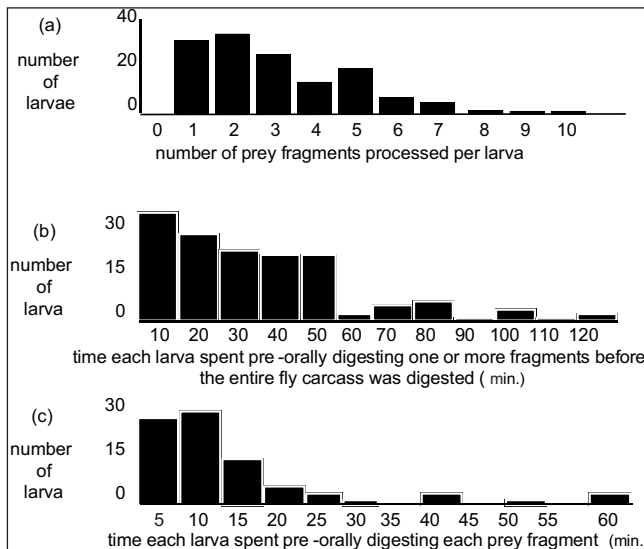


Fig. 3. Histograms of larval labor. a) Number of prey fragments processed per larva per nest. Most larvae processed at least three carcass fragments. b) Total time each larva spent digesting fragments until the entire fly carcass was digested. Most larvae labored less than 50 minutes digesting a carcass. c) Mean time each larva spent digesting each prey fragment. Most larvae labored less than 10 minutes per fragment.

colony members has evolved a step further. Larvae digest but do not ingest prey until it is fed to them by workers that distribute the product throughout the colony – larvae and adults alike.

Primitively, ants are not believed to use trophallaxis to distribute prey to larvae (Wheeler, 1910). Whole prey are brought into the nest and distributed directly to larvae (Buschinger, 1973 as cited in Hölldobler and Wilson, 1990). Larvae dissolve and consume the whole prey as they develop much as bee larvae dissolve and consume honey and pollen as they develop inside their cell (Wilson, 1971; Hölldobler and Wilson, 1990; Pereboom et al., 2003). Here we find a more advanced prey rendering process in which workers invest considerable time rendering whole prey into pieces before transporting prey tissue to the larvae (see also, *Solenopsis invicta*: Ricks and Vinson 1972, Petralia et al. 1980; Glancey et al., 1981). In addition, larvae have actually dissociated digestion and ingestion, so that most of the food they digest is taken by workers to distribute to other colony members including themselves, workers, queens and younger larvae.

These findings lend support to the idea that larvae are not passive recipients of nutrition, but rather a specialized caste that functions to digest prey tissue for consumption by other colony members (Wheeler, 1918; Went et al., 1972; Hölldobler and Wilson, 1990; Cassill and Tschinkel, 1999c). The digestive division of labor between workers and larvae is distinct from mutualistic associations found in some ants and termites, in which mutualistic fungal and microbial partners produces enzymes that digest plant tissues (Martin, 1987, 1992, Bignell, 2000). We suggest that digestive division of labor is common among higher ants and that the presence and morphology of a larval food basket will predict which ant taxa use the same mechanism as *P. spadonia*. The degree of cooperation between worker and larva reported in this study is an example of the binding forces that generate complex colony-level organization from simple interactions between individuals.

In the final analysis, our findings demonstrate a strong cooperative exchange of labor between larvae and workers for acquiring and digesting protein. Foragers retrieve prey tissue from a hostile environment. Workers inside the nest then butcher the prey into small fragments. Larvae do the work of pre-orally digesting prey tissue for distribution among colony members. All together, our study on the cooperative sharing of pre-orally digested protein and the possible sharing of digestive enzymes extends, in an incremental fashion, the mechanistic and evolutionary explanations for the social organization found in some Hymenoptera (Wilson, 1971; Hunt, 1994).

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